IN THE UNITED STATES PATENT AND TRADEMARK OFFICE

Application of:	Srivastava *et al.*	Confirmation No.:	1802
Application No.:	09/873,403	Art Unit:	1643
Filed:	June 4, 2001	Examiner:	Christopher H. Yaen
For:	COMPLEXES OF ALPHA (2) MACROGLOBULIN AND ANTIGENIC MOLECULES FOR IMMUNOTHERAPY	Attorney Docket No: (CAM No.	8449-178-999 708584-999177)

DECLARATION OF DR. KENNETH PARKER UNDER 37 C.F.R. § 1.132

Commissioner for Patents
P.O. Box 1450
Alexandria, VA 22313-1450

Sir:

I, Kenneth Parker, do declare that:

1. I am a citizen of the United States of America, and a permanent resident of Massachusetts residing at 69 Clinton St, Hopkinton.

2. I presently hold the position of Instructor at Brigham and Women's Hospital having a place of business in Boston, MA.

3. I received the degree of Ph.D. in Biochemistry from Harvard University.

4. My academic and technical experience and honors, and a list of my publications are set forth in my *curriculum vitae*, attached hereto as Exhibit 1.

5. I have read and understood the invention described and claimed in the above-identified United States Patent Application No. 09/873,403 ("the '403 application"). I have read and understand the Office Action dated November 03, 2006 ("the Office Action") and the reference Bizik *et al.* (Int J Cancer. 1986, 37(1):81-88; "Bizik") cited by the Examiner.

6. I understand that claims 1, 7-9, 40, 42, 50-57 and 61-64 of the '403 application are subject to a rejection wherein the Examiner contends that these claims lack novelty over Bizik.

7. I understand that claims 1, 7, 40, 42, 50, 51, 54, 55, 61, and 63 of the '403 application recite a purified molecular complex comprising an alpha (2) macroglobulin (α2M) polypeptide noncovalently associated with an antigenic molecule which displays the antigenicity of an antigen overexpressed in a cancer cell relative to its expression in a noncancerous cell of said cell type.

8. I understand that claims 8, 9, 52, 53, 56, 57, 62, and 64 of the '403 application recite a purified population of molecular complexes which are at least 65% noncovalent complexes of (i) an α2M or α2M polypeptide, and (ii) an antigenic molecule.

9. I understand that the Examiner contends that Bizik discloses the purified complexes or purified population of complexes referred to in the claims cited above in Paragraphs 7 and 8. In particular, I understand that the Examiner contends that the ammonium sulfate precipitation procedure described in Bizik on pages 81-82 results in the purified complexes and purified population of complexes of the claims. For the reasons explained below, it is my judgment and opinion that the purification procedures disclosed by Bizik do not result in the purified complexes or purified population of complexes of the claims.

10. Bizik teaches the purification of α2M from the conditioned medium of a cancer cell line using a series of steps including ammonium sulfate precipitation followed by size-exclusion (molecular sieve) chromatography, preparative SDS-polyacrylamide gel electrophoresis ("SDS-PAGE"), and electroelution from the relevant gel slice (see Bizik, paragraph spanning pages 81 and 82; and Figure 1 on page 82). In the Office Action dated November 3, 2006, the Examiner contends that the ammonium sulfate precipitation step provides the purified complexes of the claims.

11. I will now discuss why neither the ammonium sulfate precipitation step, nor the combination of ammonium sulfate precipitation with size exclusion chromatography

provides the purified complexes or purified population of complexes of the claims. One skilled in the art typically construes the term "purified protein" to mean a protein that has been separated substantially from other proteins, where "separated substantially" in this context means such that, at a minimum, the protein of interest is the predominant protein by weight in the preparation. This meaning of purified is consistent with its usage in the application (see, *e.g.*, the specification at page 8, *l.* 27 to page 9, *l.* 5). Bizik teaches the purification of α2M from conditioned medium using a series of steps consisting of ammonium sulfate precipitation followed by size-exclusion chromatography, preparative SDS-PAGE, and electroelution (see Bizik, paragraph spanning pages 81 and 82; and Figure 1 on page 82). Bizik shows the progress of the purification procedure by SDS-PAGE as shown in Figure 1 (see Bizik, Figure 1 on page 82). Figure 1 of Bizik shows an SDS-PAGE analysis of a protein sample taken following the ammonium sulfate precipitation step (lane 1), a protein sample taken following the size-exclusion chromatography step (lane 2), as well as a protein sample following preparative SDS-PAGE and electroelution (lane 3) (see Bizik, Figure 1 on page 82). Lane 1 of Figure 1, which analyzes the preparation after the ammonium sulfate precipitation, clearly shows that most signal intensity in the lane is located in a portion of the lane that is not the location of the migration of α2M (see Bizik, Figure 1 on page 82). There is not even a visible α2M band in lane 1 of Figure 1.

Moreover, Bizik itself states that bovine albumin was the most abundant of the ammonium sulfate-precipitated proteins (see Bizik, page 83, col. 2). Thus, lane 1, which represents the result of the ammonium sulfate precipitation procedure, shows that α2M resulting from such a procedure would not be considered purified, because it clearly is only a minor species in the preparation. Lane 2 of Figure 1 shows a smear of proteins obtained from peak 1 of the size-exclusion chromatography step, and, in my judgment and opinion, the signal intensity at the location of migration of α2M is clearly less than half of the total signal intensity in the

lane, thus showing that α2M is not the predominant protein by weight in lane 2 of Figure 1 (see Bizik, Figure 1 on page 82). In my judgment, which I believe would be consistent with that of others skilled in the art, lane 2 may show a protein preparation "enriched for" α2M, but not a purified α2M, in view of the standard meaning of the term "purified". Indeed, the statements in Bizik are consistent with the foregoing conclusion. Bizik clearly is presenting only the α2M eluted from the preparative SDS-polyacrylamide gel slice, as shown in lane 3 of Figure 1, as containing purified α2M (see Bizik *et al.*, page 83, col. 2, ¶¶ 1 to page 84, col. 2), not that shown in lanes 1 and 2. This is appropriate since α2M in lane 3 of Figure 1 appears to be the predominant protein by weight, and there is no visible contamination by other proteins (see Bizik, Figure 1 on page 82). As discussed above, since α2M appears to be a minor component among the proteins present in the protein preparation produced by the ammonium sulfate precipitation step, alone or in combination with the size exclusion chromatography step, in my judgment and opinion, one of skill in the art would conclude that neither the ammonium sulfate precipitation step nor the size exclusion chromatography step taught in Bizik resulted in purified α2M complexes. Thus, neither the ammonium sulfate precipitation step nor the size exclusion chromatography step of Bizik produce the purified complexes or purified population of complexes of the claims.

12. I declare further that all statements made in this Declaration of my knowledge are true and that all statements made on information and belief are believed to be true and further that these statements are made with the knowledge that willful false statements and the like so made are punishable by fine or imprisonment, or both, under Section 1001 of Title 18 of the United States Code and that such willful false statements may jeopardize the validity of the application or any patent issuing thereon.

Dated: February 28, 2007

Kenneth C Parker

Kenneth Parker, Ph.D.

Attachments:

Exhibit 1: *Curriculum Vitae* of Kenneth Parker, Ph.D.

BIOGRAPHICAL SKETCH

Provide the following information for the key personnel and other significant contributors in the order listed on Form Page 2.
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NAME	POSITION TITLE
Ken Parker, PhD	Instructor, Harvard Medical School
eRA COMMONS USER NAME kcp2	

EDUCATION/TRAINING *(Begin with baccalaureate or other initial professional education, such as nursing, and include postdoctoral training.)*

INSTITUTION AND LOCATION	DEGREE *(if applicable)*	YEAR(s)	FIELD OF STUDY
Dartmouth College, New Hampshire	B.S.	1977	Chemistry/Biology
Harvard University, Cambridge, MA	Ph.D	1984	Biochemistry and Molecular Biology
Harvard Medical School, Boston, MA	Post-Doc	1984-1985	Immunology and Protein Biochemistry
Harvard University	Post-Doc	1985-1990	Immunology and Protein Biochemistry

A. Positions and Honors

Positions and Employment

1990-1997 Senior Staff Fellow, Laboratory of Molecular Structure, National Institutes of Allergy and Infections Disease, NIH
1997-2004 Discovery Proteomics and Small Molecule Research Center, PerSeptive Biosystems, acquired by Perkin Elmer, then merged with Applied Bioscience
2004-2005 Scientist, Proteomic Bioinformatics, MDS Sciex, a joint venture with Applied Biosystems
2005 Consultant, Proteomic Bioinformatics, Brigham and Women's Hospital and Children's Hospital Informatics Program, Harvard Medical School
2006 Proteomic Bioinformatic Specialist and Instructor, Brigham and Women's Hospital, Harvard Medical School, Boston, MA

Honors and Awards:

1985-1987 Fellow of the Leukemia Society of America

Patents

1. Parker, KC 1984
2. ChemPlex Methods and Apparatus for Mass Fingerprinting of Biomolecules (Submitted 12-07-2000) SYP-155.
3. Process for Analyzing Protein Samples (Submitted 5-8-2001) SYP-172.

B. Selected Peer-Reviewed Publications

1. **Parker KC**, Strominger JL. Sequence of human beta-2-microglobulin: A correction. Mol.Immunol. 1982;19:503-504.
2. **Parker KC,** Strominger JL. Localization of the sites of iodination of human beta-2-microglobulin: quaternary structure implications for histocompatibility antigens. Biochemistry 1983;22:1145-1153.
3. **Parker KC**, Strominger JC. Subunit interactions of class I histocompatibility antigens. Biochemistry 1985;24:5543-5550.
4. Murre C, **Parker KC**, Reiss CS, Driller JP, Wiley DC, Burakoff SJ, Seidman JG. Biochemical and functional analyses of a secreted H-2Ld molecule. Mol.Cell.Biol. 1986;6:1315-1319.
5. **Parker KC**, Wiley DC. Overexpression of native human beta-2-microglobulin in Escherichia coli and its purification. Gene 1989:83:117-124.

6. Silver ML, **Parker KC**, Wiley DC. Reconstitution by MHC-restricted peptides of HLA-A2 heavy chain with beta-2-microglobulin in vitro. Nature 1991;350: 619-622.
7. **Parker KC**, Silver ML, and Wiley DC. An HLA-A2/beta-2-microglobulin/peptide complex assembled from subunits expressed separately in Escherichia coli. Mol Immunol 1992;29:371-378.
8. **Parker KC**, Carreno BM, Sestak L, Utz U, Biddison WE, Coligan JE. Peptide binding to HLA-A2 and HLA-B27 isolated from E. coli: Reconstitution of HLA-A2 and HLA-B27 heavy chain / beta-2-microglobulin complexes requires specific peptides. J Biol Chem;1992;267:5451-5459.
9. **Parker KC**, Dibrino M, Hull L, Coligan JE. The $beta_2$-microglobulin dissociation rate is an accurate measure of the stability of MHC class I heterotrimers and depends on which peptide is bound. J.Immunol 1992;149:1896-1904.
10. **Parker KC**, Bednarek MA, Hull LK, Utz U, Cunningham B, Zweerink HJ, Biddison WE, Coligan JE. Sequence motifs important for peptide binding to the human MHC class I molecule, HLA-A2. J Immunol 1992;149:3580-3587.
11. DiBrino M, **Parker KC**, Shiloach J, Knierman M, Lukszo J, Turner RV, Biddison WE, Coligan JE. Endogenous peptides bound to HLA-A3 possess a specific combination of anchor residues that permit identification of potential antigenic peptides. Proc Natl Acad Sci USA 1993;90:1508-1512.
12. DiBrino M, Tsuchida T, Turner RV, **Parker KC**, Coligan JE, Biddison WE. HLA-A1 and HLA-A3 T cell epitopes derived from influenza virus proteins predicted from peptide binding motifs. J Immunol 1993;151:5930-5935.
13. **Parker KC,** Bednarek MA, Coligan JE. Scheme for ranking potential HLA-A2 binding peptides based on independent binding of individual peptide side-chains. J Immunol 1994;152:163-175.
14. DiBrino M, **Parker KC**, Shiloach J, Turner RV, Tsuchida T, Garfield M, Biddison WE, Coligan JE. Endogenous peptides with distinct amino acid residue motifs bind to HLA-A1 and HLA-B8. J Immunol 1994;152:620-631.
15. **Parker KC**, Turner RV, Coligan JE, Biddison WE. Pocket mutations of HLA-B27 show that anchor residues act cumulatively to stabilize peptide binding. Biochemistry 1994;33:7736-7743.
16. DiBrino M, **Parker KC**, Margulies DH, Shiloach J, Turner RV, Biddison WE, Coligan JE. The HLA-B14 peptide binding site can accommodate peptides with different combinations of anchor residues. J. Biol. Chem. 1994;269: 32426-32434.
17. Knierman MD, Coligan JE, **Parker KC**. Peptide fingerprints after partial acid hydrolysis: Analysis by matrix-assisted laser desorption / ionization mass spectrometry. Rapid Commun. in Mass Spec. 1994;8:1007-1010.
18. Tsuchida T, **Parker KC**, Turner RV, McFarland HF, Coligan JE, and Biddison WE. T cell responses to human myelin protein-derived peptides. Proc Natl Acad Sci USA 1994;91:10859-10863.
19. Malnati MS, Peruzzi M, **Parker KC**, Biddison WE, Ciccone E, Moretta A, Long EO. Peptide specificity in the recognition of MHC class I by natural killer cell clones. Science 1995;267:1016-1018.
20. **Parker KC,** M. Shields, M. DiBrino, Brooks A, and Coligan JE. Peptide binding to MHC class I molecules: Implications for antigenic peptide prediction. Immunol. Res. 1995;14:34-57.
21. DiBrino M, **Parker KC**, Margulies DH, Shiloach J, Turner RV, Biddison WE, Coligan JE. Identification of the peptide binding motif for HLA-B44, one of the most common HLA-B alleles in the Caucasian population. Biochemistry 1995;34:10130-8.
22. **Parker KC**. (In press) Epitope prediction for class I MHC molecules , chapter 10 in MHC Molecules: Expression, Assembly and Function, Editors Robert Urban and Roman Chicz, Landes Bioscience Publishers, Georgetown, Texas.
23. Wizel B, Houghten RA, **Parker KC**, Coligan JE, Church P, Gordon DM, Ballou WR, Hoffman SL. Irradiated sporozoite vaccine induces HLA-B8-restricted cytotoxic T lymphocyte responses against two overlapping epitopes of the Plasmodium falciparum sporozoite surface protein 2. J Exp Med. 1995;182:1435-1445.
24. Alexander-Miller MA, **Parker KC**, Tsukui T, Pendleton CD, Coligan JE, Berzofsky JA. Molecular analysis of presentation by HLA-A2.1 of a promiscuously binding V3 loop peptide from the HIV-1 envelope protein to human CTL. International Immunity 1996;8: 641-649.
25. Peruzzi M, **Parker KC**, Long EO, Malnati M. Peptide sequence requirements for recognition of HLA-B27 by specific NK clones. J Immunol 1996;157: 3350-3356.
26. Honma K, **Parker KC,** Becker KG, McFarland HF, Coligan JE, Biddison WE. Identification of an epitope derived from human proteolipid protein that can induce autoreactive CD8+ cytotoxic T lymphocytes restricted by HLA-A3: evidence for cross-reactivity with an environmental microorganism. J Neuroimmunol 1997;73:7-14.

27. Zappacosta F, Borrego F, Brooks AG, **Parker KC**, Coligan JE. Peptides isolated from HLA-Cw*0304 confer different degrees of protection from natural killer cell-mediated lysis. Proc Natl Acad Sci U S A 1997;94:6313-6318.
28. Templeton TJ, Fujioka H, Aikawa M, **Parker KC**, Kaslow DC. Plasmodium falciparum Pfs40, renamed Pf39, is localized to an intracellular membrane-bound compartment and is not sexual stage-specific. Mol Biochem Parasitol. 1997;90: 359-365.
29. Hines WM, **Parker KC**, Peltier J, Patterson DH, Vestal ML, Martin SA. Protein identification and protein characterization by high-performance time-of-flight mass spectrometry. J Protein Chem 1998;17:525-6.
30. **Parker KC,** Garrels JI, Hines W, Butler EM, McKee AH, Patterson D, Martin S. Identification of yeast proteins from two-dimensional gels: working out spot cross-contamination. Electrophoresis 1998;11:1920-1932.
31. Garvin AM, **Parker KC**, Haff L. MALDI-TOF based mutation detection using tagged in vitro synthesized peptides. Nat Biotechnol 2000;18: 95-97.
32. Zappacosta F, Tabaczewski P, **Parker KC,** Coligan JE, Stroynowski I. The murine liver-specific nonclassical MHC class I molecule Q10 binds a classical peptide repertoire. J Immunol 2000;164:1906-1915.
33. Griffin TJ, **Parker KC**, Han DKM, Gygi SP, Rist B, Lee H, Aebersold R. Toward a High-Throughput Approach to Quantitative Proteomic Analysis: Expression-Dependent Protein Identification by Mass Spectrometry. J Am Soc Mass Spectrom 2001;12:1238-1246.
34. **Parker KC.** Scoring Methods in MALDI Peptide Mass Fingerprinting: ChemScore and the ChemApplex Program. J Am Soc Mass Spectrom 2002;13:22-39.
35. Malmstrom J, Larsen K, Malmstrom L, Tufvesson E, **Parker KC**, Marchese J, Williamson B, Patterson D, Martin S, Juhasz P, Westergren-Thorsson G, Marko-Varga G. Nanocapillary liquid chromatography interfaced to tandem matrix-assisted laser desorption/ionization and electrospray ionization-mass spectrometry: mapping the nuclear proteome of human fibroblasts. Electrophoresis. 2003;24:3806-14.
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41. Hattan SJ, Parker KC Methodology utilizing MS signal intensity and LC retention time for quantitative analysis and precursor ion selection in proteomic LC-MALDI analyses Anal Chem 2006;78:7986-7996.